MICROBIAL RESISTANCE AND BIOCIDES

A review by the International Scientific Forum on Home Hygiene (IFH)

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FORWARD

The following document has been produced by the International Scientific Forum on Home Hygiene based on a detailed review of the scientific literature relating to microbial resistance to antibiotics and biocides. The aim was not only to understand what is known about the relationship between exposure to biocides and reduced sensitivity to antibiotics and biocides, but to attempt to clarify the practical implications for the use of biocides in the domestic environment. This paper refers only to the use of biocides with the aim of preventing cross contamination and cross infection in the home, and not to the medical use of biocides to reduce carriage of pathogens on human skin, including medicated soap.

CONSENSUS STATEMENT OF THE IFH

The development of microbial resistance to antibiotics and the threat this represents to antibiotic use in clinical practice is a real concern. The general conclusion by the scientific and medical community is that the main cause of the problem is inappropriate use of antibiotics in clinical practice – although use of antibiotics in veterinary medicine and agricultural feedstuffs may also be involved. A number of scientists have also considered the possibility that use of biocides could be an additional contributory factor. At a meeting of the IFH Scientific Advisory Board in May 1999 current scientific data regarding microbial resistance to biocides and antibiotics was assessed in order to evaluate whether:

- Biocide use has, or could have, an impact on antibiotic resistance in clinical practice
- Biocide use encourages, or could encourage, the development of microbial resistance to biocides
- Whether there are practical implications that indicate the need for a change in policy on biocide usage in the domestic setting.

The following represents the consensus statement of the IFH board, based on currently available scientific data:

- Whilst laboratory studies have shown potential links between the development of reduced susceptibility to certain types of biocides and the development of reduced susceptibility to antibiotics under certain conditions, there is no evidence that biocide use has been a significant factor to date in the development of antibiotic resistance in clinical practice – antibiotic misuse is the most significant causative factor.
- It must be borne in mind that as increasing antibiotic resistance continues to reduce our ability to treat certain infections, then infection prevention through good hygiene – not only in hospitals but also in the community – becomes of even greater importance.
- Since biocides, used responsibly, form an integral part of infection prevention through good hygiene, not only in hospitals but also in the home, it is important to address concerns that use of biocides may contribute to the development of biocide resistance in practical use. Although laboratory studies provide evidence that prolonged exposure to low levels of certain biocides can be associated with reduced microbial susceptibility, this decreased susceptibility is small relative to concentrations of biocides used in practice. There is currently no evidence to suggest that biocide usage at its current levels (i.e., in domestic and other settings) compromises the effectiveness of hygiene procedures under in-use conditions.

- It is important to recognise that, by reducing the number of infection outbreaks through effective hygiene, the number of antibiotic courses prescribed can be lowered, which can in turn reduce the impact of antibiotic resistance, i.e., as part of a responsible hygiene policy correct biocide use can contribute to controlling the impact of antibiotic resistance. Thus, the possible risks associated with reduced susceptibility to antimicrobials must be weighed against the risks of not using disinfection where hygiene cannot be achieved by other means.

- Education and advice on these issues is important so that health professionals and the public recognise the important benefits of infection prevention through good hygiene and are more fully informed about the possible threats from antibiotic misuse.

Laboratory studies have shown that bacteria possess mechanisms whereby a link between biocide exposure and the development of biocide and antibiotic resistance can exist. For this reason it is important to ensure that biocides are used responsibly as part of a good hygiene routine in the domestic setting in order to avoid the possibility of any impact on antimicrobial resistance in the future:

- they should not be used indiscriminately and irresponsibly*

- when used they should, wherever possible, be used at concentrations and under conditions which give rapid and effective inactivation of micro-organisms

- they should be used in a way that as far as possible avoids the build-up of residues of biocide which might encourage the selection of resistant strains

It is important to continue to research and to monitor these issues.

* The IFH is currently producing a paper which gives guidelines as to where the use of biocides is considered advisable and beneficial in the home as a means to prevent infection and cross contamination.

CONTENTS

1. INTRODUCTION

Microbial resistance to antibiotics and the threat that this represents in clinical practice is a real and increasing concern. It is generally accepted that the main cause of the problem is inappropriate usage and over-prescribing of antibiotics – although use of antibiotics in agricultural feedstuffs and veterinary practice may also be involved (Anon 1997; Rao 1998; Anon 1999a; Feinman 1999; Magee *et al.* 1999). Concern about bacterial resistance has led to calls for increased education on the correct use of antibiotics and more stringent infection control measures to reduce the transmission of infection (Anon 1998; 1999b,c; Smith *et al.* 1999).

A number of scientists have expressed concern that biocide use may also be a contributory factor in the development of antibiotic resistance (Stickler and Thomas 1980; McMurry *et al.* 1998; Russell *et al.*1998, 1999a; Levy 1998). Some laboratory studies have demonstrated such a link but there is currently no evidence that it is a significant factor in the development of antibiotic resistance in clinical practice. This is supported by the fact that the history and pattern of biocide use does not correlate with emergence of antibiotic resistance; in the UK, biocide use in hospitals has declined over the last 30 years whereas antibiotic resistance has steadily increased. Indications are that, if biocides do have some contributory role, it is likely to be minor - but this aspect requires constant review. Looking from another perspective, it should be noted that if reducing the number of infections through effective hygiene is important, then it is also important to ensure that biocide use, as an integral part of good hygiene practice, is not discouraged in situations where there is real benefit in terms of preventing infection transmission. This means that it is also necessary to assess the possibility that biocides used indiscriminately could contribute to the development of biocide resistance which could compromise their in-use effectiveness.

In this review the literature regarding the mechanisms whereby bacteria may become less sensitive to biocide action is evaluated, before looking at the links between antibiotic and biocide resistance and their implications. A glossary at the end of the document provides a brief explanation of some of the more technical terms used.

2. REDUCED MICROBIAL SUSCEPTIBILITY TO BIOCIDES

The scientific literature documenting cases of reduced susceptibility to biocides and the mechanisms responsible has been comprehensively reviewed (Brown *et al.* 1990; Gilbert *et al.* 1990; Russell 1998; McDonnell and Russell 1999; Russell *et al.* 1999a). When assessing the data it is noteworthy that much of the terminology and methodology relating to biocide resistance derives from work on antibiotics and there is a tendency to extrapolate from one situation to the other without considering the fundamental differences which exist between the mechanisms of action of antibiotics and biocides, and the methods used to evaluate the efficacy of each. Care must be taken in interpreting the data, especially regarding the following:

1. ‘Resistance’ is a relative word. In the context of antibiotic use, susceptibility tests are used by clinicians to predict the likelihood of therapeutic success. Where there is a change in susceptibility that renders an agent ineffective against an infection, previously treatable by that agent, the organism is referred to as ‘resistant’. By contrast, much of the work investigating ‘resistance’ to biocides and other inimical agents such as heat, irradiation etc. is laboratory-based and there is currently little evidence from clinical situations. Examples illustrating reduced susceptibility to biocides and other inimical processes do not necessarily correlate with failure of the product or process, i.e., failure to achieve disinfection or antisepsis. Thus, the term ‘resistance’ when applied to these changes can be misleading. It is suggested that the term ‘reduced susceptibility’ may be more appropriate in this context.

2. Antibiotics often exert their effect through growth inhibition caused by inactivation of a single target, and achieve bacterial eradication in conjunction with the humoral and cellular defence mechanisms of the host. The efficacy of antibiotics is assessed using the minimum inhibitory concentration (MIC) which has direct relevance because it can be related to blood and serum levels. Biocides, on the other hand, most often have multiple cell targets and their efficacy and benefit is dependent for the most part on producing effects that cause rapid kill – expressed as the $\log_{10}$ reduction in bacterial numbers. In many cases, however, conclusions about so-called 'resistance' to biocides are based on MIC determinations that reflect activity against the most sensitive target site and have little relevance in assessing whether this value is correlated to reduced kill or product failure.

3. MECHANISMS BY WHICH BIOCIDE EXERT THEIR ANTIMICROBIAL ACTION

Studies of the mode of action of antimicrobial agents suggest that, unlike antibiotics for which selective action against specific cell targets is fundamental to their clinical value, biocides may act at one or several sites within the cell wall, membrane or cytoplasm. Studies of the mode of action of biocides are reviewed in detail by Russell and Chopra (1996), Denyer and Stewart (1998) and Russell *et al*. (1999b).

For some agents, such as polymeric biguanides, disruption of the cell wall is an integral part of their action (Wilkinson and Gilbert 1987); by destabilising cations associated with the cell envelope, thereby causing reorganisation of lipopolysaccharide (LPS), these agents self-promote their entry to the cell, where they exert their lethal action. For many biocidal agents used as disinfectants and antiseptics, most particularly those which exhibit surface active properties, such as the quaternary ammonium compounds, phenols and substituted phenols, and polymeric biguanides, indications are that their bactericidal action results from generalised disruption of the cell membrane. For chemically reactive biocides such as chlorine and oxygen-releasing agents, bactericidal action most probably results from oxidation of thiol and other groups in a whole range of membrane-bound and intracellular enzymes whilst agents such alcohol and chlorhexidine at bactericidal concentrations produce denaturation of cytoplasmic proteins and coagulation of cell contents.

Studies with some of these compounds suggest that at high concentrations, as used in practice to achieve rapid microbiocidal action, they produce generalised effects such as disruption of the cell membrane or inactivation of a broad range of enzymes, but at lower, growth inhibitory concentrations they may act in much the same way as antibiotics, specifically affecting one or two cellular targets. However, even for biocides that affect multiple targets, susceptibility of each target is likely to be variable and dependant on the concentration of the biocide.

Examples of biocides with specific cellular effects include the membrane-active phenolic agents such as tetrachlorsalicylanilide and Fentichlor, which act as uncouplers of oxidative phosphorylation at concentrations that inhibit cell growth (Bloomfield 1974; Hamilton 1968). Phenoxyethanol (and its analogues 2,4 dichlorphenoxyethanol (dichlorophen) (Gilbert *et al*. 1978) also affect a multitude of intracellular targets, dependent on concentration (Gilbert *et al*. 1977a,b). At sub-lethal biocide levels a variety of concentration-dependant inhibitory processes take place. These range from action as a potassium:proton antiporter and respiration uncoupler, to competitive inhibition of NADH-binding by malate dehydrogenase. DNA biosynthesis is slowed relative to general anabolism in the cell at sub-MIC (Gilbert *et al*. 1977b). Isothiazolone biocides also have a more complex effect on cellular machinery than is generally accepted. Whilst their primary action on the cell is mediated through a chemical interaction with thiols (Collier *et al*. 1990a) specificity is seen towards a number of respiratory enzymes (Collier *et al*. 1991) and, at sub-MIC levels, they have been shown to induce filamentation in both bacteria and the fission yeast *Shizosaccharomyces*

pombe (Collier *et al*.1990b). Whilst lethal doses affect thiol groups on proteins, sub-lethal doses more specifically affect DNA (Gilbert *et al*. 1980) and bacterial cell walls (Collier *et al*. 1990b). Phenylethanol is known to inhibit the initiation of DNA replication and to cause a similar filamentation to that caused by the isothiazolones (Silver and Wendt 1967). Most recently studies have shown that, in *Escherichia coli* and *Mycobacterium smegmatis,* triclosan has a specific action on the enzyme enoyl reductase which is essential for fatty acid synthesis at concentrations which are growth inhibitory (McMurry and Levy 1998, McMurry *et al.* 1999).

4. MECHANISMS WHICH REDUCE MICROBIAL SUSCEPTIBILITY TO BIOCIDES

There appear to be a variety of mechanisms that account for the wide range of sensitivities which different types of micro-organisms exhibit when assessed by sensitivity assays such as MICs and minimal bactericidal concentrations (MBCs). Some organisms are intrinsically resistant, a natural property of all strains of the organism or genus. Alternatively, they may undergo changes in susceptibility which reflect either the conditions under which they were cultivated, mutation at a sensitive target site, or the acquisition of genetic elements which encodes resistance mechanisms. In contrast to antibiotic resistance, where acquired bacterial resistance is a more significant problem because it can cause rapid development and spread of resistance to multiple antibiotic agents, for biocides intrinsic mechanisms are judged to be of more practical relevance (Russell 1998; McDonnell and Russell 1999).

4.1 Intrinsic properties of bacteria conferring reduced susceptibility to biocides

Biocides interact with micro-organisms initially at the cell surface. Intrinsic susceptibility is thus significantly influenced by cell wall composition and components of the outer surface, which determine this interaction and subsequent uptake by the cell. As summarised in Tables 1 and 2, Gram-negative bacteria are generally relatively less susceptible to biocides than Gram-positive bacteria because their cell walls present a more significant barrier to entry. The outer membrane of Gram-negative bacteria acts as a permeability barrier because the narrow porin channels limit the penetration of hydrophobic molecules and the low fluidity of the LPS leaflet slows down the inward diffusion of lipophilic compounds. *Mycobacteria*, which possess a waxy envelope that inhibits the uptake of some biocides, are generally even more resistant (Russell 1996). The coat and cortex of bacterial spores present a barrier to biocide entry explaining their relatively extreme insusceptibility. When spores germinate, the biochemical and structural changes that follow often results in the germinating cells becoming more susceptible to the action of some biocides.

Table 1. Relative microbial susceptibility to biocides

Enveloped viruses	Most susceptible
Gram-positive bacteria	
Large non-enveloped viruses	
Fungi	⇓
Gram-negative bacteria	
Mycobacteria	
Spores	
Coccidia	
Prions	Least susceptible

Adapted from Russell (1999)

It is not always the case that Gram-negative bacteria are more resistant to biocides than Gram-positive bacteria. For example, chlorine is more active against *P. aeruginosa* and *Proteus mirabilis* than against *Staphylococcus aureus* (Russell *et al*. 1999b).

Recent studies have shown that efflux pumps, sometimes with unusually broad specificity (towards mostly lipophilic or amphipathic molecules), also contribute to the intrinsic resistance of Gram-negative bacteria by pumping out a variety of agents, including dyes, detergents and antibiotics. In the context of antibiotic resistance, the term multidrug resistance (Mdr) is used to describe a situation where reduced susceptibility to an antibiotic is associated with reduced susceptibility to other chemically unrelated antibiotics through a common efflux mechanism. In *Pseudomonas aeruginosa* the MexAB, MexCD and MexEF efflux systems and in *Escherichia coli* the AcrAB efflux systems act as a transporter for a whole range of biocides and antibiotics. Schweizer (1998, 2000) suggests that, for *P. aeruginosa,* the presence of the Mex efflux systems coupled with the narrow porin channels in the outer membrane of the cell which restricts diffusion of antimicrobial agents into the cells is responsible for the very high intrinsic resistance of this species to antimicrobial agents compared with other Gram-negative species. As discussed in more detail in section 6.1.1 Mdr systems are also implicated in development of reduced antibiotic and biocide susceptibility.

As well as impaired uptake or increased efflux, some micro-organisms demonstrate intrinsic resistance through inactivation of biocides (Table 2). Biocides are less likely than antibiotics to be inactivated by bacteria but examples include the inactivation of phenols and some aldehydes in species of *Pseudomonas* (Sondossi *et al.* 1986). Inactivation of quaternary ammonium compounds (QACs), chlorhexidine, and phenylethanol has also been reported, but only at concentrations below those used in practice and is therefore unlikely to be a mechanism of resistance to these compounds (Russell *et al.* 1999b). Recently Nishihara *et al.* (2000) have reported the inactivation of didecyldimethyl ammonium chloride by a strain of *Pseudomonas fluorescens*, whilst detoxification of triclosan has been reported by Meade (2000).

Table 2. Intrinsic mechanisms which reduce susceptibility of bacteria to biocides

Type of resistance	**Examples**	**Mechanisms of resistance**
1. Impermeability		
Gram-negative bacteria	• QACs • Triclosan • Diamidines	Barrier presented by outer membrane preventing biocide uptake; glycocalyx may also be involved. Efflux pumps
Mycobacteria	• Chlorhexidine • QACs • Glutaraldehyde	Waxy cell wall prevents adequate biocide entry May be associated with mycoylarabinogalactam in *M. chelonae*
Bacterial spores	• Chlorhexidine • QACs • Phenolics	Spore coat and cortex present a barrier to biocide entry
Gram-positive bacteria	• Chlorhexidine	Glycocalyx/mucoexopolysaccharide may be associated with reduced biocide diffusion
2. Inactivation (chromosomally mediated)	• Chlorhexidine	e.g., Breakdown of chlorhexidine molecule may be responsible for resistance

4.1.1 Reduced susceptibility to biocides resulting from phenotypic changes

The intrinsic sensitivity of an organism to a biocide, documented from MIC or MBC determinations using standard methods, is by no means a 'fixed value'. The cell phenotype expressed can vary significantly depending on the environmental conditions under which it is grown. Physiological (phenotypic) adaptation of micro-organisms that reduces susceptibility to biocides in response to environmental changes is also considered as intrinsic resistance.

The effects of nutrient depletion in inducing significant levels of resistance to antimicrobial agents including antibiotics have been well documented (Brown and Williams 1985; Brown *et al*. 1990; Gilbert *et al.* 1990; Brown and Barker 1999). The resistance of these micro-organisms to both biocides and antibiotics may derive partly from changes in outer cell layers that increase the barrier properties and prevent access to their site of action, but other changes are also involved. The association of micro-organisms with solid surfaces leads to the formation of a biofilm, with bacteria in different zones of the biofilm experiencing different nutrient environments and displaying different physiological properties (Gilbert *et al.* 1990; Brown and Gilbert 1993; McDonnell and Russell 1999). Reduced susceptibility of bacteria in biofilms to antimicrobials can sometimes be extreme and is probably caused by a variety of factors including nutrient depletion within the biofilm, reduced access of the biocide to cells in the biofilm, chemical interaction between the biocide and the biofilm, and the production of degradative enzymes and neutralising chemicals (Brown and Gilbert 1993). Studies by Maira-Litran *et al.* (2000a,b) suggest that the expression of the gene *mar*, and both *mar*-dependant and *mar*-independent upregulation of *acr*, is insufficient to account for the ciprofloxacin resistance of *E. coli* biofilms. Brown and Barker (1999) review studies which show intracellular grow in macrophages or protozoa is also associated with reduced susceptibility to biocides and antibiotics.

The impact of changes in antimicrobial susceptibility associated with environmental factors is discussed in more detail in section 6.3.

4.2 Reduced susceptibility to biocides associated with genotypic changes (acquired mechanisms)

Reduced susceptibility to biocides may also be acquired through mutation, or by the acquisition of a plasmid or transposon.

4.2.1 Plasmid-mediated mechanisms

The first evidence that plasmids can encode reduced susceptibility to biocidal agents involved metals such as silver and mercury, and the organomercury compounds. Bacterial resistance to mercury is plasmid-borne, inducible and may be transferred by conjugation or transduction (Russell 1997). Silver salts are important topical antimicrobials but most metal compounds that illustrate plasmid-mediated resistance are not widely used as disinfectants. More recent studies (reviewed by Russell 1997, 1998) have also demonstrated the presence of plasmids in bacteria with low level resistance to chlorhexidine, QACs, triclosan, and hexachlorophene as well as reduced susceptibility to the less important diamidines, acridines and ethidium bromide. However, apart from the studies detailed below, the physiological basis of the reduced susceptibility was not determined and there is no specific evidence that the plasmid was actually involved.

Plasmid-mediated changes in susceptibility to biocides have been studied most extensively in *S. aureus* where it has been shown that some biocides, based on their MICs, are less inhibitory in these organisms. Reduced susceptibility to ethidium bromide, acriflavine, QACs such as cetrimide and benzalkonium chloride (BAC), and diamidines such as propamide isethionate (PI) is mediated by a

group of structurally related plasmids encoding *qac* genes (Tennent *et al.* 1989; Littlejohn *et al.* 1992; Reverdy *et al.* 1993; Behr 1994; Paulsen *et al.* 1996; Mitchell *et al.* 1998).

Table 3. *qac* genes and susceptibility of *S. aureus* strains to some biocides.

	MIC ratios*		
qac gene	CH	CTAB	BAC
qacA	2.5	4	>3
qacB	1	2	>3
qacC	1	6	>3
qacD	1	6	>3

CH: Chlorhexidine diacetate

CTAB: Cetryltrimethylammonium bromide

BAC: Benzalkonium chloride

*Ratios are MICs for strains of *S. aureus* carrying various *qac* genes divided by the MIC for a strain carrying no gene.

Adapted from McDonnell and Russell (1999)

The *qacA* gene is located mainly on members of the pSK1 family of multiresistance plasmids and codes for a multidrug protein efflux pump. *qacB* is found on beta-lactamase and heavy metal resistance plasmids such as pSK23, and although coding for a similar protein, specifies reduced susceptibility only to intercalating dyes and quaternary ammonium compounds. *qacC* and *qacD* encode resistance to ethidium bromide and some quaternary ammonium compounds and is typically found on the plasmids pSK89 or pSK41. Leelaporn *et al*. (1994) also report reduced susceptibility to biocides such as BAC and chlorhexidine in coagulase-negative staphylococci that was mediated by the plasmid-encoded genes *qacA* and *qacC* previously characterised in *S. aureus*.

Reduced susceptibility to biocides mediated by plasmids can also occur through a number of other mechanisms. Plasmid-mediated resistance to inorganic and organic mercury compounds is through inactivation, although inactivation of other biocides mediated by plasmids has not been reported. Reduced uptake mediated by plasmids may be responsible for silver resistance although silver-resistant bacteria can also accumulate high silver concentrations, indicating the existence of other resistance mechanisms.

Evidence of plasmid-borne resistance determinants in Gram-negative species that code for resistance to biocide molecules used as antiseptics and disinfectants is relatively limited and is reviewed by Russell (1997). As far as Gram-negative species are concerned plasmid-encoded changes in outer membrane proteins associated with decreased susceptibility to formaldehyde has also been shown in *E. coli* and *Serratia marcescens* (Kaulfers *et al.* 1987). The presence of a plasmid in *E. coli* was shown to be associated with alterations in the composition of the outer membrane lipopolysaccharide and reduced numbers of porins, which thus reduced outer membrane permeability to cetrimide and other agents (Rossouw and Rowbury 1984).

4.2.2 Mutational resistance to biocides

Antibiotics usually act at specific sites within the bacterial cell. Therefore, a mutation that alters the target site is likely to cause resistance. Chromosomal gene mutations conferring resistance to antibiotics are relatively well studied. Mechanisms include bypass of a sensitive step, alterations in the normal target site of the antibiotic such as a change in the bacterial ribosome or a metabolic enzyme, or overproduction of the target enzyme or of an efflux pump.

Some biocides also known to have specific target sites have been identified, but by contrast there are relatively few studies of the role of mutation in conferring resistance. Recent investigations however now show that mutations which affect the AcrAB and MexAB multidrug efflux pumps in *E. coli* and *P. aeruginosa* respectively are associated with reduced susceptibility to some biocides (Moken *et al.* 1998; McMurry *et al.* 1998; Schweizer 1998; Chuanchuen *et al.* 2000). Following the identification of enoyl reductases as the site of action of triclosan in *E. coli* and *M. smegmatis* (McMurry and Levy 1998; McMurry *et al.* 1999) it has also been shown that mutants lacking this enzyme show reduced susceptibility to triclosan which is seen as an increase in MIC. They proposed that, like antibiotics, triclosan has a specific mechanism of action by blocking lipid synthesis for which enoyl reductase is an essential enzyme. *S. aureus* mutants with enhanced resistance to triclosan (>1 ug/ml) have also been isolated (Suller and Russell 2000). In all cases however the mutants were not less sensitive to the bactericidal effects of triclosan than the parent wild type strain. Similar results were also obtained for *E. coli* by McDonnell and Pretzer (1998) who concluded that triclosan, similar to other biocides, has multiple targets and that, at bactericidal concentrations triclosan produces additional cellular changes to which both the wild type and the mutant are equally sensitive. These findings and their implications are discussed in more detail in section 6.1.1.

5. POSSIBLE LINKS BETWEEN ANTIBIOTIC RESISTANCE AND REDUCED SUSCEPTIBILITY TO BIOCIDES

A large number of studies have been carried out to evaluate whether clinical or environmental isolates that show reduced susceptibility to biocides also exhibit resistance to antibiotics. Alternatively, these same studies have looked for reduced susceptibility to biocides in antibiotic resistant isolates. Although some laboratory findings suggest that the development of biocide and antibiotic resistance can be associated, other studies indicate no such link. Some of these studies are reviewed in the following sections. This subject has also been reviewed by Russell *et al.* (1986), Russell *et al.* (1998, 1999a,b), and McDonnell and Russell (1999).

5.1 Examples of studies showing reduced susceptibility to biocides in antibiotic-resistant bacteria

Stickler and Thomas (1980) tested the MICs of Gram-negative bacteria from the hospital environment for a range of antiseptics, disinfectants and antibiotics and found that approximately 10% of the isolates (mainly *Pseudomonas, Proteus, Providencia*) exhibited some reduced susceptibility to chlorhexidine and cetrimide and were also generally more resistant to multiple antibiotics.

Reverdy *et al*. (1992) showed that antibiotic-sensitive *S. aureus* and other staphylococci are usually antiseptic-sensitive, whereas strains for which MICs indicated intermediate or high antiseptic resistance were also more resistant to a wide variety of antibiotics. Increased MICs for methicillin-resistant *S. aureus* (MRSA) strains have been reported for some biocides including chlorhexidine, cetrimide, benzalkonium chloride (BAC), hypochlorite, triclosan, parahydroxybenzoates and

betadine (Townsend *et al.* 1983; Brumfitt *et al.* 1985; Mycock 1985; Yamamoto *et al*. 1988; Al-Masaudi *et al*. 1988, 1991a; Cookson *et al.* 1991a; Irizarry *et al*. 1996; Mitchell *et al.* 1998). Although Cookson *et al*. (1991b) isolated a clinical strain of MRSA which showed resistance to triclosan and mupirocin, by contrast, Bamber and Neal (1999) found that, out of 16 MRSA isolates exhibiting low-level mupirocin resistance none had increased MICs to triclosan. Suller and Russell (2000) have shown that acquisition of a plasmid encoding mupirocin resistance was not associated with changes in triclosan MICs. Suller and Russell (1999) found that a series of MRSA clinical isolates showed low-level reduced susceptibility to a range of biocides including chlorhexidine, cetylpyridinium chloride (CPC), BAC and triclosan, as compared with methicillin-sensitive *S. aureus* (MSSA) strains when evaluated both by bacteriostatic (MIC) and, for chlorhexidine and CPC, bactericidal assays. The fact that several of these studies showed that strains with elevated MICs remain equally susceptible to the bactericidal concentrations of these agents as MSSA will be discussed in more detail later.

5.2 Examples of studies showing no change in susceptibility to biocides in antibiotic-resistant bacteria

Stecchini *et al.* (1992) showed that, despite widespread antibiotic resistance of Enterobacteriaceae strains isolated from mince meat, these were not resistant to the bactericidal activity of an amphoteric Tego disinfectant. Similarly Fernandez-Astorga *et al.* (1995) isolated psychrotrophic non-fermenting Gram-negative strains from vegetables and showed that antibiotic-resistant strains were susceptible to the bactericidal action of QAC and hypochlorite disinfectants.

Baillie *et al.* (1992) evaluated the chlorhexidine sensitivity of 33 clinical isolates of *Enterococcus faecium* sensitive to vancomycin and gentamicin with that of 12 vancomycin- and 7 gentamicin - resistant strains. The results showed no increase in resistance to chorhexidine as indicated by evaluation of MICs. Interestingly, a study of 67 ciprofloxacin-resistant isolates of *P. aeruginosa* yielded 4 isolates which were hypersensitive to chlorhexidine (MIC 5 mg/l) whilst none were found amongst 179 ciprofloxacin-sensitive isolates (Baillie *et al.* 1993).

Anderson *et al.* (1997) determined the susceptibilities of vancomycin-resistant and -sensitive enterococci (VRE and VSE) to various concentrations of commonly used hospital disinfectants, including quaternary ammonium compounds, phenolics and an iodophor, at recommended use-dilutions and extended dilutions using the suspension test. They concluded that there was no relationship between vancomycin resistance and resistance to disinfectants at use-dilution. This was confirmed by Suller and Russell (1999) who showed that a series of VRE and VSE clinical isolates showed no significant difference in sensitivity to chlorhexidine, CPC and triclosan when evaluated both by bacteriostatic (MIC) and bactericidal assays.

Bamber and Neal (1999) determined the MIC of 186 isolates of MRSA and MSSA. Published data for triclosan state that the expected MICs for staphylococci should be between 0.01 ppm and 0.1 ppm. They found 14 isolates (7.5%) with MICs greater than 1.0 ppm, but these were equally distributed between MRSA and MSSA strains.

Rutala *et al.* (1997) and Payne *et al.* (1999) showed that a series of antibiotic-resistant clinical and environmental isolates, including *P. aeruginosa, Klebsiella* species*, E. coli, S. aureus* and *Staphylococcus epidermidis*, were not less susceptible to the bactericidal activity of disinfectants, which included a phenol and a quaternary ammonium disinfectant, chloroxylenol, BAC, cetrimide and povidone iodine.

5.3 Preliminary conclusions based on the results of these studies

Overall, these studies suggest that there is no consistent pattern in the relationship between reduced biocide susceptibility and antibiotic resistance. Observations suggesting or refuting such a link vary according to the nature of the biocide and the antibiotic, and the conditions under which the evaluation was carried out. It also depends on whether the evaluation was carried out using a bactericidal or bacteriostatic assay. Assessing the implications is also made more difficult by the fact that, in many cases, there is no indication of whether the changes were stable or reversible; the stability of reduced susceptibility in biocides should always be examined. In fact it would be unbelievable if links between biocide and antibiotic resistance were not observed since changes in the outer layers of the cell, particularly of Gram-negatives where these layers act as the barrier limiting the access of antimicrobials and particularly high molecular weight hydrophobic molecules to their target sites, are likely to affect both biocides and antibiotics. The variable nature of the observable links between changes in biocide and antibiotic susceptibility suggests that there is no single underlying cause and that realistic assessments of the implications can only be made by better understanding their physiological basis. In the following sections, the various studies of the mechanisms responsible for changes in susceptibility to biocides and antibiotics are evaluated in order to assess possible practical implications.

6. PRACTICAL IMPLICATIONS OF LINKS BETWEEN BIOCIDE AND ANTIBIOTIC RESISTANCE

In assessing the implications of the observed links between biocide usage and the development of reduced susceptibility to antibiotics and biocides a number of questions require consideration.

With regard to the problem of antibiotic resistance:

1. Since some bacteria possess mechanisms encoding for both reduced susceptibility to biocides and resistance to antibiotics, is it possible that biocide use could contribute to the emergence of antibiotic multiresistant populations in clinical practice? If it is thought that biocides contribute to the development of antibiotic resistance, to what extent is this a contributing factor as compared to other factors such as antibiotic overuse?

With regard to the usage of biocides in home hygiene:

2. To what extent does exposure of microbial populations to biocides result in reduced biocide susceptibility and how does this correlate to a failure to achieve ‘hygiene’ in practical situations?

Further important considerations are:

3. What is the practical and clinical significance of changes in susceptibility to biocides and antibiotics induced by exposure to antimicrobials as compared with the reduced susceptibility induced by ‘normal’ environmental ‘stresses’?

4. Is it possible that biocides used responsibly can actually help reduce the spread of antibiotic resistance by preventing infections and therefore reducing the need for antibiotics?

These questions are considered in the following section.

6.1 Biocide usage and antibiotic resistance. Is it possible that biocide use could contribute to the emergence of antibiotic resistant populations in clinical practice?

The studies described in section 4.1 indicate that bacterial populations can simultaneously become less susceptible to both antibiotics and biocides. In theory this establishes the possibility that continuous exposure to biocides could add to, or enhance, the selective pressure exerted by antibiotic use, which is currently assumed to be the major cause of antibiotic resistance in clinical practice. Where simultaneous changes in susceptibility to antibiotic and to biocide types used as disinfectants and antiseptics have been investigated, the resistance determinants mostly involved are genes that encode for multidrug efflux pumps – either plasmid-borne in Gram-positive species or chromosomally-encoded in Gram-negatives. Some recent studies have shown that this could also arise where a selective target site for biocides is shared by a therapeutic agent or agents. Since there are fundamental differences between these aspects they will be considered separately.

6.1.1 Chromosomally-encoded multidrug efflux pumps in Gram-negative bacteria

As discussed in sections 4.1 and 4.2, chromosomally-encoded multidrug efflux pumps are key in defining the intrinsic susceptibility of Gram-negative bacteria to both biocides and antibiotics, but also play a role in the development of multiresistance. Phenotypic adaptation in response to environmental stimuli or mutations that increase expression of these efflux genes result in elevated levels of resistance.

In *P. aeruginosa,* the MexAB, MexCD and MexEF multidrug efflux systems act as transporters for a variety of agents including tetracycline, ciprofloxacin, fluoroquinolone, β-lactams, fusidic acid, etc. Mutations in the repressor genes cause overexpression of the *MexAB-OprM* operon producing increased resistance to these agents (Poole *et al*. 1993; Li *et al.* 1994a,b; Li *et al*. 1995) whilst low-level fluoroquinolones are known to select for overproducers of these pumps (Kohler *et al.* 1997). Schweizer (1998) has shown that triclosan is also a substrate for the MexAB-OprM multidrug efflux pump in *P. aeruginosa*. Further studies (Chanchuen *et al.* 2000) have shown that exposure to triclosan selected a multidrug resistant strain that hyper-expressed the *MexCD* efflux system genes from a susceptible population of *P. aeruginosa* mutants in which *MexAB* was deleted. In addition to reduced susceptibility to triclosan this strain showed a marked decrease in susceptibility, as assessed by MICs, to several antibiotics including tetracycline, ciprofloxacin and trimethoprim.

In *E. coli,* the AcrAB efflux system acts as a transporter for tetracycline, ciprofloxacin, fluoroquinolone, β-lactams and novobiocin as well as ethidium bromide, acriflavine, phenylethylalcohol, sodium dodecyl sulphate and deoxycholate (Nakamura 1968; Ma *et al.* 1993; Okusu *et al.* 1996; Buysse *et al.* 1996). Acr systems are also found in other species of Enterobacteriaceae such as *Salmonella* (Sukupolvi *et al.* 1984; Nikaido *et al*. 1998c). Recent studies (Moken *et al.* 1998; McMurry *et al.*1998) indicate that *Acr* can also act as an efflux system for triclosan, chloroxylenol and quaternary amines. These systems, together with multidrug efflux pumps identified in other bacterial species such as *Neisseria gonorrhoea, Haemophilus influenza*e and *Burkholdia cepacia*, are reviewed in more detail by Nikaido (1998a,b) and Paulsen *et al*. (1996).

In assessing the potential importance of multidrug efflux pumps, the first point to establish is their significance with regard to therapeutic failures in clinical practice. Evidence for this comes mainly from studies with clinical isolates of *P. aeruginosa*. Using *P. aeruginosa* PAO, Rella and Haas (1982) showed that mutations in the repressor gene of the *MexAB* operon leads to overproduction of this efflux system and raises the MICs of ciprofloxacin and carbenicillin 8 and 32 times, respectively, in comparison with the wildtype strain. Among carbenicillin-resistant clinical isolates of *P. aeruginosa* collected in a UK study in 1982 almost 80% did not produce carbenicillin-

hydrolysing β-lactamase and appeared to belong to the elevated efflux type (Williams *et al.*1984). A study in France found that about one third of ticarcillin-resistant *P. aeruginosa* clinical isolates had a resistance pattern characteristic of 'intrinsic resistance' (Bert and Lambert-Zechovsky 1996).

For *E. coli* and *Salmonella* the evidence for a role of multidrug resistance in clinical practice is less convincing; Ma *et al.* (1994) suggest that, although Acr and MexAB have a similar substrate range, only MexAB is likely to produce clinically significant levels of resistance to small lipophilic agents such as tetracycline, chloramphenicol and fluoroquinolones, presumably because their entry through the narrow *P. aeruginosa* porin channels is significantly slower than through *E. coli* porin channels. In *E. coli* and *Salmonella* species, Acr pumps are controlled by the positive regulator MarA (produced by the *Mar* operon). Alekshun and Levy (1997) suggest that increased production of the AcrAB system in *E. coli* occurs most readily on account of mutations in the *marR* repressor locus causing overproduction of MarA. A survey of 28 quinolone-resistant clinical strains of *E. coli* showed that elevated *Mar*A transcription did occur in 3 strains (Maneewannakul and Levy 1996). It must be borne in mind however that overexpression of *MarA* also reduces levels of a porin, OmpF, which probably contributes to the resistance of such strains (Gutman *et al*. 1985).

For some clinical strains of *Staphylococcus* species it has also been noted that significant resistance to fluoroquinolones is partly due to overexpression of the multidrug efflux *norA* gene (Yoshida *et al.* 1990; Kaatz *et al.* 1993; Ng *et al.* 1994).

However, even for *P. aeruginosa*, it is not clear what fraction of the resistant isolates of clinical origin correspond to efflux mutants and opinions differ as to their importance in clinical practice. In a recent review, Nikaido (1998a) concedes that multidrug efflux is "probably not yet the most frequent mechanism of resistance amongst clinical isolates" but notes that reports suggest efflux-based resistance now occur with increasing frequency.

Further insight into the potential significance of multidrug efflux strains can be gained by considering what their 'normal' physiological function might be. Current knowledge suggests that efflux pumps are part of the natural defence mechanisms against toxic compounds that exist in the environment. Although Gram-negative bacteria defend against large hydrophilic molecules by utilising the narrow porin channels in the outer membrane, the lipopolysaccharide-containing bilayer still allows slow diffusion of lipophilic agents. Thus, AcrAB is essential for survival of *E. coli* as it makes this species resistant to the bile salts in their normal habitat (Thanassi *et al.* 1997). The wide substrate specificity of these pumps and, for the AcrAB pump, regulation by global stress signals rather than specific substrates, make these systems well suited for a general defensive role. However, their broad spectrum probably causes as well as solves problems since they may 'accidentally' pump out key metabolites such as pyruvate or lactate (Kramer and Nickerson 1984). It is possible that efflux pumps originally evolved to allow bacterial populations to respond to changes in their environment but, faced with increasing threat from chemotherapeutic agents, they have subsequently been recruited to overcome the effects of these agents. If the primary function is to facilitate continuous 'modulation' of efflux activity in response to their ever-changing environment it seems unlikely that mutant strains in which efflux systems are constitutively expressed are the 'preferred' or stable state. When the selective pressure is removed it is suggested that mutant populations will decline in favour of wild type populations.

If one were to accept that exposure of microbial populations to low-level antibiotics is a causative factor in the emergence of multidrug efflux mutants which confer clinically significant antibiotic resistance – then the possibility that exposure to low-level biocide could have the same effect must also be considered. Recent studies by Levy and co-workers (Moken *et al.* 1997) show that mutants of *E. coli* selected for reduced susceptibility to a pine oil disinfectant also show reduced

susceptibility to multiple antibiotics including tetracycline, chloramphenicol, ampicillin and nalidixic acid, which is mediated via the *mar* and *acr* operons. Importantly, however, the level of antibiotic resistance which develops is relatively low and unlikely to compromise effectiveness in clinical use.

Realistically it may be that antibiotic efflux caused by exposure to biocides is of no particular significance in the real world, since it represents only one of a whole series of inimical agents that elicit this effect. George and Levy (1983) and Cohen *et al.* (1989) have shown that low levels of antibiotics such as chloramphenicol and tetracycline also act as weak inducers of the *MarRAB* operon in *E. coli* thereby increasing drug efflux. Additionally *MarRAB* responds to a range of inducers reflecting a variety of environmental conditions including exposure to the weak acid salicylate (Cohen *et al.* 1983). The fact that tetracycline and chloramphenicol were much less effective than salicylate (albeit at higher concentrations) in upregulation of *mar* suggests the role of salicylate as a 'normal' substrate for this pump.

If these efflux pumps indeed evolved as a defence against antimicrobial agents occurring in the environment (as suggested by Miller and Sulavick 1996) then 'natural' antimicrobial agents would also contribute to this problem. The *marRAB* regulon is also induced by the positive regulator SoxS that is produced by transcription of *soxRS* in response to exposure to free radicals. Recently it has been postulated (Dodd *et al.* 1998; Bloomfield *et al.* 1998) that if bacterial cells are growth-arrested by treatment with an inimical agent, the imbalance between anabolism and catabolism causes a burst of free radical production which results in loss of viability in addition to damage produced by the inimical process. If this hypothesis is correct it is possible that any chemical substance which produces a sudden decrease in growth rate could cause increased expression of *acr* multidrug efflux pumps. Thus far only pine oil disinfectants have been evaluated but it is suggested that further studies are likely to show that selection of *acr* efflux mutants is facilitated not only by exposure to all types of antimicrobial substances, including so-called 'natural' antimicrobials, but also many other chemical agents including various types of household surfactants and cleaning chemicals and other compounds which form part of daily life.

6.1.2 Plasmid-mediated efflux mechanisms in Gram-positive species

By contrast, plasmid-mediated antibiotic resistance in Gram-positive bacteria is well established as a significant clinical problem. Identification of plasmid-mediated biocide resistance raises concern as to whether biocide exposure could contribute to the spread of antibiotic resistance by selection and dispersal of plasmids bearing resistance determinants for antibiotics along with determinants for biocides (Russell 1997; Russell *et al.* 1999a; Stickler and King 1999). This could involve plasmids bearing a resistance determinant for a common target site shared by the antibiotic and one or more biocides. Alternatively the plasmid might bear the biocide resistance determinant alongside structurally unrelated determinants for antibiotic resistance e.g., penicillin-binding proteins.

A detailed review by Russell (1997) shows that at present staphylococci are the only bacteria that has been studied in any detail, in which the genetic basis of reduced biocide susceptibility is plasmid-mediated. Where reduced biocide susceptibility occurs in *S. aureus*, it is commonly associated with plasmid-encoded efflux proteins. Studies of *S. aureus* strains by Tennent *et al.* (1989), Littlejohn *et al.* (1992) and Reverdy *et al.* (1993), as outlined in section 4.2.1 have shown that genes encoding multidrug efflux such as *qacA,B,C* and *D,* in conjunction with antibiotic resistance determinants on multiresistance plasmids, are associated with reduced susceptibility (as assessed by MICs) to a series of biocides including ethidium bromide, acriflavine, QACs such as cetrimide and BAC, chlorhexidine and PI. Studies by Behr (1994) and Heir *et al.* (1995) show that these *qac* genes are widely distributed in clinical and food isolates of *S. aureus.* Of concern is the finding that the *qacA/B* family of genes show significant homology to other energy-dependant

transporters such as the tetracycline transporters found in tetracycline-resistant strains, whilst *qacA/B* can be borne on penicillinase plasmids (Rouche 1990; Russell 1999a).

Studies, as outlined in section 5.1, report increased MICs against plasmid-carrying MRSA strains for a range of biocides including chlorhexidine, cetrimide, benzalkonium chloride, hypochlorite, triclosan, and betadine (Townsend *et al.* 1983; Brumfitt *et al.* 1985; Mycock 1985; Yamamoto *et al*. 1988; Al-Masaudi *et al*. 1988, 1991a; Cookson *et al.* 1991a; Irizarry *et al*. 1996). Although this reduced susceptibility is thought to be predominantly plasmid-mediated, apart from the studies with GNAB plasmids and chlorhexidine detailed in section 6.1.3, the physiological basis was not elucidated. It is quite likely however that, for some of the studies involving QACs, qac efflux proteins were involved.

As with Gram-negative efflux pumps, the original physiological functions of the staphylococcal *qac* genes are also unclear but Paulsen *et al.* (1996) suggest that they evolved before the introduction and use of topical antimicrobials and disinfectants. This is based on the observation that the chronological emergence of plasmids bearing these genes in clinical isolates mirrors the introduction and usage of organic cationic compounds in clinical practice. Thus, although they may once have played other physiological roles in their original host organism, they appear to have been acquired by clinical pathogens for protection against hydrophobic antimicrobial agents and become widely disseminated due to the selective pressure imposed by the use of agents such as acriflavine, BAC, chlorhexidine and cetrimide in antiseptic and disinfectant formulations. Recently Paulsen *et al*. (1999) have presented evidence, based on DNA homology, that *qacA* has evolved from *qacB*. These authors have proposed that emergence of the *qacA* determinant among *S. aureus* clinical isolates during the 1980s may have resulted from the extensive usage of divalent cationic compounds such as chlorhexidine in hospital environments, although prospective studies of the comparative resistance of hospital isolates obtained during this period are needed to substantiate this proposal. Bacquero *et al.* (1991) found no evidence of any association between prolonged chlorhexidine usage in the hospital setting and reduced susceptibility to chlorhexidine.

For Gram-negative bacteria it is generally assumed that changes in susceptibility towards antibiotics and biocides mediated through *AcrAB* and *mexAB* multidrug efflux genes is unlikely to be transferable as it is chromosomally mediated. However, Saier *et al.* (1998) have shown that genes for divalent cation efflux pumps of similar construction already exist on plasmids and suggest that there is no reason to expect that drug efflux genes of this type could never be transferred to resistance plasmids.

6.1.2.1 Stability and transferability of plasmid-mediated biocide and antibiotic resistance

The identification of plasmids conferring reduced susceptibility to biocides (albeit low-level resistance indicated by increased MICs) together with antibiotic resistance raises the possibility that this co-resistance may be rapidly spread within microbial populations or between different bacterial strains and their persistence facilitated by the selective pressure of low-level biocide residuals in the environment.

Studies by Tennent *et al. (*1985) demonstrated transfer of recombinant *S. aureus* plasmids into *E. coli* that conferred increased MICs of cationic agents. Al-Masaudi (1991a) showed that *S. aureus* can acquire new resistance determinants through conjugation and plasmid transfer. These workers also showed however (Al-Masaudi *et al.* 1991b) that, whereas antibiotics such as gentamicin and vancomycin stimulate plasmid transfer frequency, biocides such as cationic agents and organomercurials, reduced plasmid transfer in *S. aureus*. The authors suggest that biocides affect the synthesis of the conjugative apparatus and the cell membrane, but the clinical significance of these observations requires further investigation. More recently Pearce *et al*. (1999) have studied

the effect of sub-MIC concentrations of chlorhexidine, povidone iodine and cetrimide on the acquisition of antibiotic resistance genes by conjugation and transduction. At low concentrations, cetrimide reduced plasmid transfer efficiency whilst the other biocides had no effect. Low concentrations of povidone iodine and chlorhexidine reduced phage transfer efficiency whilst cetrimide caused some increase, probably by an effect on the recipient strains.

Russell (1997) concluded that plasmid-borne mercury resistance, transferred by conjugation or transduction, is a common property of clinical isolates, but for biocides commonly used as disinfectants the level of plasmid-mediated biocide resistance *per se* is relatively low suggesting that transfer of these plasmids is of much less significance.

6.1.3 Other relevant studies involving biocides with specific target sites

The identification of selective targets for biocides such as the enoyl reductase enzyme, a target site for triclosan in *E. coli* and *M. smegmatis* (McMurry and Levy 1998), offers the possibility that these agents could select spontaneous mutant populations with reduced susceptibility to single target therapeutic agents which share the same target. Of concern also is the possibility (but no evidence) that, if the resistance determinants were transferred to plasmids bearing resistance determinants for one or unrelated targets conferring high level antibiotic resistance e.g., penicillin binding proteins, then persistent low-level biocide exposure could select for antibiotic multiresistant populations through selective pressure and plasmid transfer.

The recent finding that the enoyl reductase enzyme in *M. smegmatis* (Mcmurry *et al*. 1999) is the target not only for triclosan but also for the chemotherapeutic agent isoniazid is of concern. Its deletion was found to result in a 1.2 to 8.5 fold increase in the MIC to isoniazid and a 4 to 6.3 fold increase in MIC of triclosan. Despite earlier studies reported by Cookson *et al.* (1991b) that, although the biochemical basis of the resistance was not elucidated, identified MRSA strains for which low-level triclosan resistance could be co-transferred with mupirocin resistance to sensitive *S. aureus* recipients, recent studies of *S. aureus* (Suller and Russell 2000) have shown that the acquisition of a plasmid encoding mupirocin resistance was not associated with changes in triclosan MICs. Although mutants of *S. aureus* with enhanced resistance to triclosan (>1ug/ml) have been isolated by Suller and Russell (2000), which in several cases was stably inherited in the absence of triclosan, these mutants were not less sensitive than the parent strain (MIC of triclosan, 0.025 ug/ml) to a range of antibiotics or to the lethal effects of triclosan at 7.5 ug/ml.

Although plasmid-borne and/or stable resistance has been reported for some other biocides (for example, Tattawasart *et al*. 1999 showed development of stable resistance to chlorhexidine and cetylpyridinium chloride (CPC) in *Pseudomonas stutzeri*, which suggests that a specific target site might be involved, at the present time no such sites have been identified for these agents.

A significant number of other studies, as outlined in section 5.1, have reported increased MICs against plasmid-carrying MRSA strains for a variety of biocides including chlorhexidine, cetrimide, benzalkonium chloride, hypochlorite, triclosan, parahydroxybenzoates and betadine (Townsend *et al.* 1983; Brumfitt *et al.* 1985; Mycock 1985; Yamamoto *et al*. 1988; Al-Masaudi *et al*. 1988, 1991a; Cookson *et al.* 1991a; Irizarry *et al*. 1996). Apart from the studies with GNAB plasmids and chlorhexidine, however, the extent and the physiological basis of this resistance has not been established. Studies of MGRSA strains (*S. aureus* strains resistant to both methicillin and gentamicin) suggest that the plasmids which they contain (termed GNAB plasmids) code for a nucleic acid binding (NAB) site which is a common target for gentamicin and chlorhexidine. MGRSA strains without NAB plasmids were more sensitive to chlorhexidine than methicillin sensitive strains, whereas MRSA isolates with GNAB plasmids encoding resistance to gentamicin were more resistant to the bisbiguanide (Cookson et al. 1991a).

A number of other studies suggest the possibility of shared target sites although again there is no evidence of simultaneous reduced susceptibility to biocides and antibiotics, either from laboratory or clinical studies. Agents such as the aminoglycosides, for example, are known to gain access to the cell through a self-promoted mechanism (Hancock *et al*. 1981; Taber *et al*. 1987) whereby the agent destabilises cations associated with the cell envelope causing re-organisation of the LPS and facilitating antibiotic entry. It is notable that many biocides, particularly polymeric biguanides (Wilkinson and Gilbert 1987) share this mechanism of cellular uptake. Similarly phenylethanol (Silver and Wendt 1967) is known to inhibit the initiation of DNA replication and to cause a similar filamentation to that of the isothiazolones. Should these sub-lethal effects be directed towards a DNA gyrase then it is not difficult to imagine how the action of quinolone antibiotics might also be affected.

6.1.4 Preliminary conclusions based on the results of these studies

A review of the laboratory-based data detailed in this section leads to the conclusion that some types of biocides do have the potential to encourage the emergence of antibiotic-resistant populations, either by selection of multidrug resistant populations or, in Gram-positives, by transfer of multiresistant plasmids. At present however there is no evidence that this phenomenon occurs in clinical practice or in the general environment.

In assessing the implications of the currently available data, two criteria need to be assessed, firstly how extensively these mechanisms might occur in the environment or in clinical practice and secondly whether the level of antibiotic insusceptibility is sufficient to compromise clinical effectiveness. Indications are that the latter is likely to differ according to the nature of the antibiotic, the target site and the biocide involved. Current laboratory-based evidence suggests that for antibiotic-resistant populations involving the Gram-negative efflux pumps, the levels of antibiotic resistance which are induced or acquired are relatively low and unlikely to compromise clinical effectiveness. The significance of multiresistant plasmid transfer in Gram-positive species, particularly in *Staphylococcus* species, requires further investigation as does the possibility of mutation in shared target sites in both Gram-negative and Gram-positive species. However if acquisition of multi-target plasmids encoding reduced biocide susceptibility alongside antibiotic resistance determinants is a real possibility, it is interesting to speculate whether this is likely to occur to any extent outside the hospital environment. Logically the sequential addition of antibiotic resistance determinants onto plasmids containing determinants conferring reduced susceptibility to biocides should only occur in environments where microbial populations are not only exposed to a persistent low levels of the biocide in question but are also regularly subjected to the selective pressure from a series of different antibiotics. Such a situation is more likely to occur in the hospital than in the home where antibiotic usage (and perhaps, until recently, also biocide usage) is much less. Interestingly, Jarvinen *et al.* (1993) reported a study of clinical isolates of *Streptococcus mutans* from the mouths of 114 school children and students from families in which about 70% used oral preparations containing chlorhexidine on a regular basis. The results showed no evidence of increased resistance to chlorhexidine or to a range of antibiotics as tested using MICs.

It must be borne in mind that, despite the fact that biocides are normally used at concentrations which are rapidly bactericidal, in any environment (or downstream of that environment) there is likely to be a continuum of biocide concentration ranging from treatment concentration to nil. Theoretically, there will be sub-lethal concentrations of biocide for any given cellular target at some point along this concentration gradient, providing a selection pressure for mutations in a multiplicity of cellular targets. Biofilm communities also provide highly selective environments where sharp gradients of antimicrobial agents will prevail and selective pressures will be greatest (McBain *et al*. 2000).

Since selection or transfer of determinants for reduced susceptibility will only apply to biocides which have selective target sites it seems unlikely (although but not impossible) that it could occur with chemically-reactive agents such as chlorine or oxygen-releasing agents, or with solvent molecules such as alcohols. This likelihood is further reduced by the fact that these agents are unstable or volatile and thus do not persist in the environment in an active form. It is thus perhaps surprising that Murray *et al*. (1984) reported that chlorination of sewage produced an increase in the proportion of antibiotic resistant isolates. A suggested explanation was that chlorination caused selection of stress-tolerant strains which were also antibiotic resistant, or stimulated transfer of resistance plasmids, but these aspects were not investigated.

6.2 Biocide usage and biocide resistance. Does reduced susceptibility to a biocide result in preferential survival and spread of biocide-resistant strains in practice?

The studies described in section 4 show that exposure to low levels of biocides can also be associated with reduced biocide susceptibility of microbial populations. In many cases however the reduced susceptibility is again seen as an increase in the MIC. An important issue is whether this is commensurate with loss of efficacy *in vivo* since, in practice, disinfectants and antiseptics are used at much higher concentrations which have a rapid microbicidal action as shown by bactericidal suspension test methods and sometimes also *in vivo* tests. Some insights can be gained by reviewing those studies where reduced susceptibility was evaluated based on biocidal activity as well as MICs.

Cookson *et al.* (1991a) found that whereas the MIC of chlorhexidine against MRSA clinical isolates was higher (4-8 µg/ml) than for MSSA isolates (0.37-2µg/ml), there was no significant difference in the efficacy of chlorhexidine against the resistant and sensitive strains when inoculated onto the arms of volunteers and evaluated by a bactericidal assay. A clinical isolate of MRSA which showed an elevated MIC when challenged with triclosan (2-4µg/l compared with 0.12µg/l for other MRSA isolates) showed no loss of sensitivity to triclosan when measured in a bactericidal assay (Cookson *et al.* 1991a). Essentially, similar findings were reported by Suller and Russell (1999, 2000).

Using triclosan McDonnell and Pretzer (1999) found that whereas the MIC for *FabI* mutants of *E.coli* was increased to 25-50µg/ml compared with 0.1µg/ml for the wild type strain, tests using triclosan-containing products showed no differences in the rate of kill of the mutants when compared to wild-type strains. This is consistent with the hypothesis that triclosan has multiple target sites within the bacterial cell such that mutations which affect the growth inhibitory properties of triclosan are unlikely to have any impact on susceptibility to biocidal concentrations.

Heir *et al.* (1995) reported that 13% of staphylococcal isolates from a food manufacturing environment had MIC values for BAC between 4 and 11mg/l compared with 70% of remaining isolates which had MICs of less than 2mg/l. The resistance was generally low level and suspension tests showed that recommended use concentrations produced a 5 log reduction in viable count. Plasmid-borne *qac* genes were identified in a number of the food-associated staphylococci. In a study of *Pseudomonas* species isolated from poultry carcasses these workers also showed that, out of two strains deemed resistant to BAC as determined from their MICs (MIC>200µg/ml), only one was shown to be resistant when evaluated by the suspension test (Langsrud and Sundheim 1997a). A more recent study by Heir *et al.* (1999) showed that *S. aureus* cells expressing a plasmid-borne *qacG* had a survival advantage (reduced log kill) in environments containing low concentrations of BAC compared to QAC-sensitive controls. However, a 5 log reduction in viable counts was obtained at BAC concentrations well below recommended user concentrations.

Despite this, however, studies are sometimes reported of situations where biocides are in constant use and where clinical or environmental isolates show reduced susceptibility to the biocidal activity

of the agent or product to an extent which could compromise in-use effectiveness. Understanding whether extensive biocide usage could be the cause, however, depends on understanding whether the reduced susceptibility is phenotypic in nature (induced by exposure to biocide or other environmental factors – see section 6.3.2) and thus reversible - or stable and acquired - which for many of these studies was not properly established. Some of these reports are summarise as follows.

Disinfectants based on QACs, and chlorine-based products are widely used in the food industry. Several reports have described reduced susceptibility in environmental or other isolates to these compounds under conditions relating to practical usage, especially among Gram-negative species. Higginbottom *et al*. (1964) reported a higher occurrence of isolates resistant to hypochlorite after changing sterilisation practice from steam to the use of chlorine compounds in dairy farms but the nature or the stability of the resistance was not investigated.

By contrast, Mead and Adams (1986) investigating *S. aureus* strains isolated from turkeys and turkey products found that chlorine concentrations of 1mg/l produced a 4 log reduction when tested against strains isolated immediately after slaughter, but only a 2 log reduction when tested against endemic strains colonising processing equipment. These results were confirmed by Bolton *et al.* (1988), but these workers showed that this resistance was primarily due to their ability to grow in clumps and produce an extracellular slime layer that acts either as a physical barrier or as a chlorine inactivator thereby reducing the kill rate.

Similarly, Sundheim *et al.* (1998) found that more than 40% of lactobacilli strains isolated from packed meat survived exposure to 200 mg/l BAC, with some strains surviving the lowest recommended in-use concentration of commercial products. No plasmid linkage could be identified and again the authors suggested that the resistance derived from the production of exopolysaccharides by the lactobacilli that act as a physical barrier preventing uptake.

Pseudomonads are generally found to be more resistant to QACs than staphylococci and studies by Langsrud and Sundheim (1997a) showed that approximately 30% of *Pseudomonas* isolates from poultry carcasses were able to grow at concentrations of 200µg/ml BAC. These workers recognised that selection of intrinsically resistant strains through the constant usage of BAC could be the cause, but adaptation could equally be the explanation. This latter is supported by the fact that the resistance was reversible. Langsrud and Sundheim (1997b) showed that resistance developed during the lag phase before growth commenced and in the presence of relatively low concentrations of BAC, but was lost within 4-8h following removal of the BAC. From the speed of the adaptation the authors concluded that it was probably a regulated process. This could also account for the fact that the resistance was not seen in bactericidal assay.

In the USA, Willingham *et al*. (1996) tested the bactericidal activity of 350 isolates from commercial chicken hatcheries for resistance to commercial preparations of QACs, phenolics and glutaraldehyde. Nineteen isolates (including *Serratia marcescens*, *Bacillus* species, *Enterococcus* species and *P. stutzeri*) from 2 of 3 hatcheries were resistant to disinfectant at and above the recommended use concentration and exposure time. Some isolates were multiresistant but only 3 showed resistance to QACs compared with 7 for phenol and 15 for glutaraldehyde. The authors suggested that this may be correlated with the usage of glutaraldehyde in US hatcheries over many years. No investigations were carried out to determine whether the resistance was reversible although some stability is suggested by the fact that all isolates were passed at least once through tryptone soya medium to produce the challenge inoculum.

Stickler *et al.* (1971) studied the effect of repeated antiseptic usage on the bacterial flora of the urethral meatus in patients undergoing intermittent bladder catheterisation. They examined the bacterial flora after daily washing with aqueous chlorhexidine (600µg/ml) from the date of injury to

the time they developed urinary tract infection. Interestingly they found that the initially Gram-positive, sensitive flora was usually replaced by a Gram-negative flora less sensitive to chlorhexidine. More importantly they found that some of the resistant strains (mainly *Proteus mirabilis, P. aeruginosa, Providencia stuartii* and *Klebsiella* species) had MICs of 200 up to 800µg/ml, well above the level of 10-50µg/ml usually reported for Gram-negative species.

Later, Stickler and Thomas (1980) tested a large collection of Gram-negative isolates from a variety of clinical and hospital settings to a array of antiseptics and disinfectants including chlorhexidine, cetrimide, glutaraldehyde and a phenolic formulation. The general conclusion was that antiseptic and disinfectants resistance is not a widespread phenomenon in species responsible for UTI. They found that approximately 10% of the isolates (mainly *Pseudomonas, Proteus, Providencia*) exhibited some resistance to chlorhexidine but these came from situations where there was extensive use of chlorhexidine. Further investigations with *Prov. stuartii* showed no evidence of a plasmid link (Stickler *et al*. 1983) and the authors concluded that the resistance was most likely an intrinsic property induced by persistent exposure to the biocide. Dance *et al*. (1987) isolated an antibiotic resistant clinical stain of *Pr. mirabilis* responsible for a hospital outbreak. This isolate was resistant to the growth inhibitory action of chlorhexidine at 800mg/l alongside resistance to multiple antibiotics but again there was no evidence of a genetic link.

Suller and Russell (1999) showed that a series of MRSA clinical isolates had low-level resistance, as evaluated by MICs to chlorhexidine, CPC, BAC and triclosan as compared with MSSA strains. For chlorhexidine and CPC, but not triclosan, this resistance was correlated with decreased susceptibility as evaluated by bactericidal assays but again this was low level and the assays were carried out at concentrations well below normal use concentrations. Stepwise exposure to increasing concentrations of chlorhexidine, CPC and triclosan produced some increase in the MIC, but the resistance was unstable again indicating that it was intrinsic rather than acquired in nature.

Most recently Tattawasart *et al*. (1999) showed that strains of *P. stutzeri* and *P. aeruginosa* developed resistance to chlorhexidine and CPC when exposed to increasing concentrations which, for *P. stutzeri* but not *P. aeruginosa,* was stable. Reduced sensitivity following exposure to chlorhexidine was also demonstrated by bactericidal assays, although use concentrations were not tested. Attempts to transfer chlorhexidine resistance from *P. stutzeri* to *P. aeruginosa* by conjugation were not successful and the authors suggest that a likely reason for the co-resistance is a non-specific decrease in cell permeability. The fact that the resistance of *P. stutzeri* was stable through 15 subcultures suggests a possibility that the decrease in cell permeability arose by selection of mutants with altered cell wall structure (Tattawasart *et al.* 2000a,b).

In the clinical environment, Nagai and Ogase (1990) isolated strains of *Achromobacter xylosoxidans* from a 0.4% chlorhexidine solution hand-washing reservoir with an MBC more than 10-fold higher than the chlorhexidine solution in the reservoir. In this case the resistance was not plasmid-mediated, and again it seems reasonable to consider that the bacteria adapt themselves gradually to the disinfectant over prolonged contact.

6.2.1 Preliminary conclusions based on the results of these studies

The overall conclusion based on current evidence, as also expressed by Russell (1997) and Russell *et al.* (1999a), is that although development of reduced susceptibility to biocides in response to biocide exposure can occur, it is not a significant consideration in practical terms since again the level of resistance expressed is low and unlikely to compromise practical effectiveness where much higher concentrations are used. For the most part, clinical or environmental isolates obtained from environments where biocides were in constant use and where the biocide insusceptibility was demonstrated at, or above, in use concentration, the authors have concluded that the resistance

probably resulted from phenotypic adaptation, most probably related to changes in the permeability of the outer cell walls. If this is the case then it can be deduced that the resistance is not permanent but a temporary response and can be reversed by withdrawal of the biocide. For these compounds it would seem that the practice of rotation of biocides in food manufacturing and pharmaceutical manufacturing industries may now have some scientific basis despite the doubts which are raised (Murtough *et al.* 2000).

6.3 Antimicrobial resistance caused by exposure to environmental stresses

In assessing the implications of antimicrobial resistance (both to biocides and antibiotics) associated with biocide exposure, it is important that the possible impact in practical and therapeutic terms is assessed in relation to the significant changes in antimicrobial susceptibility which result from exposure of microbial populations to environmental stresses. In the following section biocide and antibiotic resistance associated with exposure to biocides is compared with that which occurs in response to environmental stress. The term 'environmental stress' is normally used to describe exposure to inimical agents (physical or chemical) which have adverse effects on the cell causing cessation of growth or loss of viability. It must also be borne in mind that any sudden change in the environment to which cells cannot immediately respond (e.g. plenty as well as starvation) could be regarded as a 'stress'.

6.3.1 Acquisition of resistance to antibiotics

Based on the current evidence presented in this paper, it seems that intrinsic and acquired antimicrobial resistance occurring in response to biocide exposure is not a significant problem *per se*. Various workers have raised concerns, however, that persistent exposure, particularly to low levels of biocides which could cause the acquisition and/or expression of resistance determinants coding even for low biocide resistance, will encourage the survival of bacterial populations in environmental and clinical situations for longer periods, thereby increasing the chances of accumulating mutations or plasmids which produce high level stable antibiotic resistance (George 1996; Nikaido 1998a,b; Russell and Maillard 2000). Currently there is no evidence to support this; for antibiotics it is known that the presence of resistance mechanisms can contribute to long-term selection and enhanced survival of resistant strains *in vivo* although it is by no means clear why reduced susceptibility to antibiotics *per se* should confer an environmental survival advantage. It is not known whether low-level resistance to biocides provides a similar survival advantage.

In assessing this possibility it is important to remember, as discussed in section 5.1.1, that low-level resistance resulting from expression of efflux pumps such *acr* or *mex*, which could enhance survival, is just as likely to occur in response to other chemical agents or exposure to environmental stress. As discussed previously it is now known that expression of *acr* (via *MarRAB* and *SoxRS*) can also occur in response to agents such as salicylate and paraquat as well as some biocides and antibiotics, and it seems likely that further investigations may show that induction can result from exposure to a whole range of antimicrobial substances and other chemical compounds in daily use. It is also known that not only chemical agents but other global 'stress' conditions such as addition of 0.5M NaCl or 4% ethanol or entry into the stationary phase (many of which represent the 'typical' environment for microbial cells in the real world) also increase expression of the *AcrAB* operon (Ma *et al.* 1995) although this effect is not mediated by *MarRAB* or *SoxRS*.

Concerns about conditions that could facilitate the acquisition of antibiotic resistance determinants through persistence of microbial populations must therefore be considered not just in relation to biocides, but also the whole series of environmental stresses to which microbial populations are continuously subjected. It is likely that bacteria are intermittently or continuously under much stress of both the general type (e.g. paucity of nutrients) and the specific type (presence of superoxide, peroxide and antibacterial substances – both endogenous and exogenous).

6.3.2 Intrinsic resistance to antibiotics and biocides through phenotypic adaptation

Changes in antimicrobial sensitivity resulting from biocide exposure are most usually established through laboratory tests in which the sensitivities of laboratory grown strains, pre- and post-biocide exposure, are compared by determining MICs or microbicidal concentrations. Such methods form the basis of the majority of the investigations described in section 4. As recently reviewed by Brown and Barker (1999), whereas studies using populations grown under optimal laboratory conditions give valuable information, in reality such conditions rarely occur *in situ*. In the domestic, as in all environments, microbes typically exist in a nutrient-depleted, slow-growing or non-growing state, either in suspension, as biomasses or adherent biofilms. Biofilms occur on the surface of meat in the domestic kitchen, or on the toilet bowl surface, or the surface of cleaning cloths. In these environments protozoa can also act as reservoirs of microbes. In clinical situations cells may grow, again under nutrient-depleted conditions, as biofilms on epithelial surfaces or intracellularly in macrophage or gut cells. Intracellular growth and growth under biofilm or planktonic conditions, in nutrient depleted, starved or stressed conditions gives rise to distinct phenotypes.

Phenotypes expressed by growth under these conditions are typically not only significantly more resistant to biocides and antibiotics, but the clinical or therapeutic impact of this reduced susceptibility may be as great, if not greater, than that associated with biocide exposure. Laboratory investigations have shown that the resistance of bacteria in the stationary phase of growth or in biofilms to many antibiotics and biocides may be orders of magnitude higher than that of log phase cells (Brown *et al.* 1990; Gilbert *et al.* 1990*;* Widmer *et al.* 1991; Evans *et al.* 1991). Laboratory studies simulating in-use conditions using *P. aeruginosa* and *S. aureus* showed that, whereas the bactericidal concentration of BAC (producing 5 log reduction in 5 min) against lab-grown suspensions was 10-20 mg/l, the concentration required to produce a similar effect against simple biofilms grown for 24 h on stainless steel discs was as much as 2000mg/l (Sims 1998; Bloomfield and Sims 1996). Interestingly, however, under the same conditions the bactericidal concentration of alcohol was little affected. For *Legionella pneumophila*, replication in macrophages results in morphological and biochemical changes and x1000-fold increases in resistance to antibiotics and biocides compared with cells grown in conventional media (Barker *et al.* 1995). Marrie and Costerton (1981) showed that cells of *Burkholdia cepacia* and *Serr. marcescens* adsorbed onto glass surfaces were able to survive in the presence of in-use concentrations of chlorhexidine (0.5 up to 2%), isothiazolones and QACs. Stickler *et al.* (1989, 1991) showed that mixed biofilms of *Citrobacter diversus, P. aeruginosa* and *Enterococcus faecalis*, and biofilms of *E. coli* grown on silicone discs showed no significant loss of viability when treated with chlorhexidine 0.2% and povidone iodine (1% w/v available iodine) over 120 min as compared with planktonic cells grown in samples of urine which showed a 2-3 log reduction. Berkelman *et al.* (1984) showed that contamination of povidone iodine formulations (1%w/v available iodine) implicated in hospital bacteraemias resulted from the formation of resistant *Pseudomonas* biofilms on surfaces of pipe work of the manufacturing equipment.

A recent study by Foley *et al.* (1999) suggests that in cells of non-sporulating bacteria in stationary phase, the general stress response results in the formation of resting or dormant cells resistant to numerous physical and chemical agents. These authors suggest that the response to stress may be a critical event in chronic infection, resulting in at least a subpopulation of cells contributing to the characteristic antibiotic resistance of chronic infections. These workers have produced evidence of strong expression of *rpo*S, the major regulator of the general stress response, in sputum from cystic fibrosis patients with chronic *P. aeruginosa* lung infection. It is suggested that the general stress response may also at least partially explain the exceptional antibiotic resistance of biofilms. It may also be of critical importance in the *in vivo* response of environmental strains to biocides. Greenaway *et al.* (1999a,b) have recently shown that the stringent response may also be a factor in

determining increased intrinsic resistance to biocides and antibiotics. They propose that the rapid increases in levels of ppGpp which result from the growth inhibitory effects of antimicrobials in turn control increased expression of *rpo*S and expression of *rpo*S-controlled resistance determinants.

It is suggested that, although the practical implications of reduced susceptibility to antimicrobial agents associated with exposure to sublethal concentrations of biocides must not be underestimated, the impact must be weighed up in relation the effects of other agents (physical or chemical) which have an inimical effect on the cells and/or the 'stressful' environmental pressures to which microbial populations are continually exposed.

6.4 Is it possible that biocides could help reduce the spread of antibiotic resistance?

In response to recent concern about the growing impact of antibiotic resistance in clinical practice (Anon 1997; Rao 1998; Anon 1999a; Feinman 1999), it is now widely accepted that more stringent measures are required to deal with this problem. Working parties across Europe and elsewhere are developing strategies aimed at reducing antibiotics in animal feeds and controlling antibiotic prescribing in humans more effectively (Anon 2000). The need for improved hygiene is recognised as a vital component of these strategies (Anon 1999a,b). By reducing the incidence of infection through improved hygiene the amount of antibiotic prescribing can be reduced, which in turn reduces the impact on antibiotic resistance. The benefits of this approach have been demonstrated in clinical settings where good hygiene has contributed to reduced antibiotic resistance through reduced prescribing (Anon 1997; Schmitz *et al.* 1998).

Overall there is good evidence to suggest that good standards of hygiene in the domestic setting, which includes not only day-to-day cleaning of the home but food hygiene, hand hygiene and hygiene related to the protection of vulnerable groups, can have a significant impact in reducing the number of infections arising in the home (Beumer *et al.* 2000). A variety of different procedures can be used to achieve hygiene in the home and in some cases this may require the use of a disinfectant or antiseptic. This being the case, it can be seen that responsible use of biocides could actually contribute to reducing the impact of antibiotic resistance. If reducing the number of infections through effective hygiene is important, then it is also important to ensure that biocide usage is not discouraged in situations where there is real benefit.

There is a need for a thorough risk-benefit analysis of the use of antimicrobial agents in the domestic setting.

7. MICROBIAL RESISTANCE AND THE HOME ENVIRONMENT

Increasingly there is evidence to show that antibiotic resistance is not only a hospital but also a community problem, although there is very little information relating specifically to the home. A recent retrospective survey of antibiotic prescribing in general practice in Wales during 1996-1998 showed a significant correlation between the incidence of antibiotic resistance in coliforms in urine samples and the patterns of antibiotic prescribing (Magee *et al*. 1999).

Control of MRSA is now recognised as a community as well as a hospital problem. Herold *et al*. (1998) and Zylke (1998) report significant increases in MRSA infections acquired in the community amongst hospitalised children without predisposing risk factors. From a 3 year audit in an NHS trust in Scotland, of 172 patents colonised with MRSA, of which 113 were community acquired, it was found that 34 had no obvious connection with hospitals (Dancer and Crawford 1999). Masterton *et al*. (1995) reported a case of MRSA carriage in a nurse associated with a hospital outbreak. Initially carriage was not eradicated by topical antimicrobial therapy and subsequent investigation showed the presence of MRSA contamination on surfaces in her home environment. Subsequent antimicrobial therapy combined with environmental decontamination was successful in eliminating this carriage.

A survey of healthy adults and children in the community conducted in 1972 showed that over 50% were found to carry antibiotic resistant coliforms in their faeces (Linton *et al*. 1972). Other studies have shown that antibiotic resistant bacteria occur in both drinking water and wastewater. A number of reports have identified cases of antibiotic resistant bacteria being brought into the home on human and animals, and transfer within the home (Allen *et al.* 1997; Cefai *et al.* 1994).

Wall *et al.* (1997) reported the health risk of multiresistant *Salmonella typhimurium* DT104 in cats. This is the second most prevalent salmonella strain after *Salmonella enteriditis* PT4 in humans and is resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines. It was found that 36% of all isolations of *Salmonella* from sick cats in the UK corresponded to this strain. Four strains had an additional resistance to trimethoprim. The authors of this report point out that since cats spend a large proportion of their time indoors close to human beings and use litter trays for defecation, there is opportunity for both adults and children to come into contact with contaminated faeces. Cats shed large numbers of *Salmonella* from the buccal cavity, which can lead to contamination of their coats.

8. CONCLUDING REMARKS

As increases in antibiotic resistance continue to reduce our ability to treat infections, then infection prevention through hygiene – not only in hospitals but also in the community – becomes of even greater importance (Anon 1998, 1999a,b; Smith *et al.* 1999). In using biocides in the home environment it is vital to ensure that product usage does not contribute to the antibiotic resistance problem. It is also necessary to ensure that the biocides which are used retain their effectiveness for use in situations where they can have real health benefits in reducing infection transmission.

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GENERAL GLOSSARY

acr	names of genes are always given in italic.
acr	the protein product of a gene is always written in roman.
Aminoglycosides	any of a group of antibiotics derived from various species of *Streptomyces* or produced synthetically.
Amphoteric	able to exhibit properties of either an acid or a base.
Amphipathic	(of a molecule) with both hydrophobic and hydrophilic properties.
Anabolism	any constructive metabolic process by which organisms convert substances into other chemical components of the organism's physical infrastructure.
Beta Lactamase	an enzyme produced by some bacteria, which is able to breakdown the active form of some penicillin antibiotics rendering them ineffective.
Beta Lactams	a group of antibiotics with a characteristic structure, including the penicillin family
Carbencillin	a semisynthetic penicillin, effective against Gram-negative bacteria.
Carbenicillin Hydrolysing beta-lactamase	a type of an enzyme (see beta lactamase) which is able to breakdown Carbenicillin (a penicillin).
Catabolism	any metabolic process by which organisms breakdown nutrients or substances into intracellular compounds, usually with the liberation of energy.
Chloramphenicol	broad-spectrum antibiotic effective against many bacteria and also rickettsiae used in the treatment of typhus, typhoid, shigellosis, and related enteric diseases.
Coagulase negative staphylococci	a species of *Staphylococcus aureus* which do not produce the coagulase enzyme.
Conjugation	sexual reproduction of bacteria, through which a donor bacterium contributes some or its entire DNA to a recipient.
Ethidium bromide	a chemical used to detect DNA after electrophoresis or in cytochemical preparations.
Fusidic acid	a fermentation product of the fungus *Fusidium coccineum* used as an antibiotic.
Genotype	the genetic composition of an organism.
Gentamicin	an antibiotic effective against a range of aerobic Gram-negative bacilli, especially the *Enterobacteriaceae* and *Pseudomonas*.
Gram negative	bacteria having a cell wall composed of a thin layer of peptidoglycan covered by an outer membrane of lipoprotein and lipopolysaccharide.

Gram positive	bacteria having a cell wall composed of a thick layer of peptidologlycan with attached teichoic acids.
Humoral	pertaining to elements dissolved in the blood or body fluids.
Inimical agent	an agent which exerts a growth inhibitory or lethal action on microbial cells.
Isoniazid	antibiotic used as a tuberculostatic, administered orally and intramuscularly.
Lipophilic	having an affinity for fat, absorbing, dissolving or being dissolved in lipids.
Lipopolysaccharide	type of membrane consisting of three parts; lipid A, the core polysaccharide and the O-specific chain. The lipopolysaccharide leaflet (LPS) of *Escherichia coli* is used in laboratory immunology.
Methicillin	used to treat infection caused by *Staphylococcus aureus*; however some strains have developed resistance (known as MRSA).
Mupirocin	an antibiotic derived from fermentation of *Pseudomonas fluorscens* effective against staphylococci.
Nalidixic acid	a synthetic antibacterial agent used orally in the treatment of urinary infections caused by Gram-negative organisms.
Novobiocin	an antibiotic obtained from *Streptomyces* species, effective chiefly against staphylococci and other Gram-positive organisms.
Operon	a segment of a chromosome comprising an operator gene and the closely linked structural gene or genes whose action it controls.
Paraquat	a poisonous dipyridylium compound whose dichloride and dimethylsulfate salts are used as contact herbicides and can be found in waste waters.
Phenotypic	the observable characteristics of an organism determined by the interaction of genes with the environment.
Plasmid	an extrachromosomal self-replicating loop of DNA found in bacterial cells.
Potassium proton antiporter	a membrane-bound active transport system, controlling potassium and proton exchange between the cell and external environment
Psychrotrophic	of a bacterium – able to grow in very cold environments.
Respiration uncoupler	a chemical which acts as an uncoupler of oxidative phosphorylation.
Salicylate	a salt of salicylic acid.
Spore (bacterial)	a refractile, oval body formed within some species of bacteria, which corresponds to a resting stage.
Streptomycin	an antibiotic effective against a wide variety of aerobic Gram-negative bacilli and some Gram-positive bacteria, including mycobacteria. Its use is limited because of the emergence of resistant strains.

Sulphonamides	antibiotics bacteriostatic against Gram-positive cocci, Gram-negative cocci, Gram-negative bacilli and a wide variety of other bacteria. They have been largely supplanted by more effective and less toxic antibiotics.
Tetracycline	an antibiotic that inhibits protein synthesis and is effective against a wide variety of organisms including both Gram-positive and -negative bacteria.
Ticarcillin	a semisynthetic penicillin bactericidal effective against both Gram- positive and -negative bacteria.
Topical antimicrobials	antimicrobials applied to a body surface.
Transduction	a method of genetic recombination in bacteria, in which DNA from a lysed bacterium is transferred to another bacterium by bacteriophage (virus specific to bacteria), thereby changing the genetic constitution of the second organism.
Transposon	a discrete DNA sequence that transposes blocks of genetic material back and forth within a bacterial cell from the chromosome to plasmids.
Trimethoprim	an antibiotic that inhibits the reduction of dihydrofolate to tetrahydrofolate, often used in combination with a sulphonamide in the treatment of urinary infections.
Vancomycin	an antibiotic which is highly effective against cocci, especially staphylococci and other Gram-positive bacteria. Used in the treatment of severe staphylococcal infections resistant to other antibiotics.

GLOSSARY OF BACTERIA

Name	**Where found**	**Action**
Achromobacter xylosoxidans	Water and human intestinal tract	Gram-negative rod shaped bacteria that can cause intestinal infections
Bacillus spp.	Widespread	Mostly non-pathogenic spore formers. Two species are of major medical importance, *B. anthracis* (anthrax) and *B. cereus* (causes food poisoning)
Burkholdia cepacia	*In damp or wet places*	Infects the lungs of patients with cystic fibrosis
Citrobacter diversus	Water, food, urine and faeces	It occasionally causes neonatal meningitis
Coliforms	Intestinal tract	Lactose fermenting spp. of family *Enterobaceriacae* often causing nosocomial and pyogenic infections, diarrhoea
Enterobacteriaceae	Soil, water, plants and animals	Family of bacteria responsible for infectious intestinal disease and opportunistic infections
Enterococci	Normal inhabitant of the human intestinal tract	Spp. of *Enterococcus* including *E. faecium* and *E. faecalis* that occasionally cause urinary tract infection, infective endocarditis, and bacteremia.
Escherichia coli	*Intestinal tract*	Some strains are members of normal gut flora, whereas other strains are pathogenic, causing gastroenteritis and diarrhoea. E.g., Entero-haemorrhagic *E. coli* is associated with haemolytic uraemic syndrome.
Haemophilus influenzae	Normal inhabitants of the upper respiratory tract, that may become pathogens	Major cause of bacterial meningitis, and may cause potentially fatal acute epiglottitis.
Lactobacilli	Widespread in nature and in the human mouth, vagina and intestinal tract	Spp. of *Lactobacillus* that use lactic acid or other end-products of fermentation as a substrate. Non-pathogen.
Legionella pneumophila	Soil, cooling tower water, shower heads, construction and excavation sites, and aerosolized droplets from heat-exchange systems	Causes legionnaires' disease and Pontiac fever.
Klebsiella spp.	Intestinal tract	Coliform that can cause nosocomial urinary, pulmonary and wound infections.

Name	**Where found**	**Action**
Mycobacteria spp.	Soil, water and living tissue such as human skin (aerobic)	Family of bacteria that contains many species including the highly pathogenic *Mycobacteria tuberculosis* and *Mycobacteria leprae* and the non-pathogen *Mycobacterium smegmatis.*
Neisseria gonorrhoea	Genitourinary tract	Causes purulent venereal discharges.
Proteus spp.	Found in faeces	Infection of the urinary tract, abdomen and wounds.
Proteus mirabillis	Most frequently isolated from human clinical material, also found in soil and sewage	Leading cause of urinary tract infections.
Providencia spp.	Found in normal urine and faeces	Potential pathogens associated with urinary tract and secondary tissue infections.
Providencia stuartii	Occurs in normal urine and faeces	Causes nosocomial infections and major agent in burnt infections.
Pseudomonas spp.	Water, soil, and decomposing material	More than hundred species, occasional pathogens that produce toxins and enzymes.
Pseudomonas aeruginosa	Urinary tract, wounds, abscesses, or the bloodstream. May also occur in the environment	Opportunistic pathogen, causes infections of skin and burns, major lung pathogen in cystic fibrosis patients.
Salmonella spp.	Widespread in animals	Enteric fevers, septicaemia, and gastro-enteritis
Salmonella enteriditis	A pathogenic species occurring in humans and in animals	Paratyphoid fever, septicaemia and gastro-enteritis.
Salmonella typhimurium	Infected humans or carriers, rats and mice	One of the most frequent agents of food poisoning, causes paratyphoid fever and diarrhoea.
Serratia marcescens	Water, soil, food and clinical material	Causes nosocomial bacteremia, endocarditis, and pneumonia in immunocompromised patients.
Staphylococcus aureus	Found in the human body	Produce toxins that cause food poisoning and toxic shock syndrome, skin infections.
Staphylococcus epidermidis	Normal human skin	Many strains are opportunist pathogens or secondary invaders in various diseases, such as abscess, infected wounds and bacterial endocarditis.
Streptococcus mutans	Human upper respiratory tract	Identified with dental caries.

GLOSSARY OF BIOCIDES

Type of Biocide	Mode or Target of Action*	Example	Uses
Acridines	DNA chelating agent	Acriflavine	Treatment of infected wounds
Alcohols	Membrane permeability	Ethanol Isopropyl alcohol Phenoxyethanol.	Ethanol: used in cosmetics as preservative IPA: surface disinfection e.g., medical wipes Phenoxyethanol: used in emulsions, e.g., eye drops etc
Aldehydes	Amino group in cytoplasm	Formaldehyde Gluteraldehyde	F: Preservative, gaseous disinfection of rooms G: Disinfection of medical equipment
Anionic Compounds	Cytoplasmic membrane	Sodium Dodecyl sulphate	Provides biocidal action for detergent products
Aromatic Diamidines		Propamide isethionate	Topical treatment for wounds
Biguanides	Coagulation and precipitation of cytoplasmic constituents	Chlorohexidine Polymeric biguanides	C: Topical antisepsis PB: sanitation of surfaces, e.g. pools
Bis-phenols	Membrane potential Electron transport chain	Triclosan Hexachlorophene Fentichlor	T: Medicated soaps, hand cleansing gels H: Surgical scrubs, medicated soaps F: topical bactericide/fungicide, on skin and mucous membranes
Chlorine Compounds	Oxidise cell components (-SH groups, nucleic acid)	Sodium hypochlorite Chlorine dioxide	Surface and water disinfection
Heavy Metal Derivatives	Electron transport chain. Thiol groups	Mercury Silver	Antiseptics/preservatives including organomercury compounds and silver salts
Iodophors	Iodination of essential molecules in cell (thiol groups)	Betadine Povidone iodine	Skin/wound disinfection
Isothiazolone	Inhibits active transport, the oxidation of glucose and also affects on thiol-containing enzymes	CIT (chlorinated isothiazolones)	Used as preservatives in cosmetics, with bacteriostatic and bactericidal properties.
Organic Salts	Uncoupling agents Proton motive force	Salicylate Parahydroxybenzoates	Salicylate: Topical skin treatments PHB: preservatives
Oxidizing Compounds	Produce hydroxyl free radicals which can attack membrane lipids, DNA and other cell components	Hydrogen peroxide Peracetic Acid	HP - disinfection of surfaces, and contact lenses PA – used in food industry, disinfection of sewage sludge
Phenols	High concentrations: disrupts cell wall and precipitates the cell proteins Low concentrations: inactivate enzymes systems and leakage of metabolites from the cell wall	Chloroxylenol Pine oil disinfectants 2-phenylphenol Deoxycholate	Chloroxylenol: Topical antisepsis Pine Oil: ingredient in disinfectants 2-PP: preservative, ingredient in pine-type disinfectants Deoxycholate: a potent fungicide
Quaternary Ammonium Compounds	Inactivation of energy-producing enzymes, denaturation of cell proteins	Cetrimide Benzalkonium chloride Cetylpyridinium chloride	Cetrimide: wound, burn, preoperative disinfection BAC: disinfectant in food hygiene CTC: skin disinfection and antiseptic treatment of wounds

*The structure and properties of biocidal agents are reviewed in Russell AD, Hugo WB and Ayliffe GAJ, eds. "Principles and Practice of Disinfection, Preservation and Sterilisation." 3rd Edition. Blackwell Scientific, 1999.